

THE MICROMORPHOLOGY OF SOIL ORGANIC MATTER - A SYNTHESIS OF RECENT RESEARCH

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INTRODUCTION

It would be appropriate to begin this review by referring to the late Professor W. L. Kubiěna, the founder of soil micromorphology, who died in 1970. In addition to his important work on the micromorphology of the main soil types and the features resulting from pedogenic processes, he laid a sound basis for work on the micromorphology of soil organic matter. By 1953, he had already described thirty distinct humus forms*. He fully appreciated the effects of microflora and fauna on humus forms and considered that every humus form showed the visible and characteristic influence of animal activity. Many of his definitions of humus forms are still used today; others have formed the basis for modifications and improvements by later workers (Jongerius, 1962; Babel, 1964, 1965, 1972b; Barratt, 1964, 1969; Zachariae, 1965, 1967a, 1967b; Bal, 1970).

This review covers the period post 1965. Since this time the use of micromorphology in soil studies has greatly increased but organic matter has received less attention than most other branches. Whereas some aspects of the micromorphology of organic matter have shown considerable progress, e.g. the development of systems of description and classification and the study of faunal activity and litter breakdown in mull and moder humus forms, others have received little attention, e.

* In the past the term 'humus form' has been used in two senses: 1. as a profile and 2. as a horizon within that profile. The term is used in this paper in the sense of Kubiěna namely as a profile. It comprises the upper part of the soil (L, F, H and A horizons) which is characteristic by the presence of organic materials which are usually in the process of being broken down by organisms.

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g. humus forms other than mull and moder, and impregnation methods. In recent years more emphasis has been given to the role of organic matter in soil structure and soil fertility and this is becoming an important field of soil micromorphology.

Description and Classification Systems

Before 1965, there was no systematic method for describing the micromorphology of humus forms and organic material. The fabric types described by Kubiěna and later by Jongerius and Barratt were already at a higher level in a classification system and there had been no attempt to provide a systematic descriptive approach on which to base the lower levels of classification. Since 1965, several descriptive systems have been evolved. A combined classification and description system by Barratt (1969) introduced a new nomenclature which for the first time avoided the confusion that had previously existed because of the use of the same terms both for humus forms and microfabrics of horizons within humus forms. Barratt's system has five main classes based on the plasma-skeleton grain concept and on the nature (mineral/organic) of the components, namely: *humiskel*, composed mainly of undecomposed organic residues; *lithiskel*, mainly of mineral grains and rock fragments; *humicol*, consisting of strongly decomposed organic residues of colloidal size; *mullicol*, an intimate mixture of decomposed organic residues and clay colloids; and *argillicol*, mainly of clay. These classes are further subdivided according to the kinds of mineral grains and organic particles, and according to structure. The system is useful for distinguishing broad differences but fails to succinctly characterize differences between related humus forms.

Two other systems of description have been drawn up, one for plant materials in various stages of decay (Babel, 1965), the other dealing mainly with faunal activity and the forms of faunal excrement (Bal, 1970).

Babel's system is based on morphological features such as plant structure, degree of birefringence and presence of coloured substances. Slightly humified plant material is characterized by recognizable plant tissues, birefringence of most

cell walls and small amounts of staining. Plant remains and birefringence decrease with humification and most material is stained with brown or black dyes. Plant remains such as xylem, parenchyma, etc. are identified as far as possible. More recently Babel (1967) has estimated amounts of different features in horizons of humus forms using an 0-5 scale.

The scheme introduced by Bal (1970, 1973) for describing organic material represents the first more or less complete basic descriptive system of the lower levels of organisation of organic soil materials. The object described, termed a humon, is defined as a collection of macroscopically and/or microscopically observable organic bodies in the soil which are characterised by a specific morphology and spatial arrangement. To study the organisation of the humon, the constituents and the way in which they are organised are described. The system has the advantage that, with that of Brewer (1964), it provides a comprehensive system for describing the organisation of undisturbed soil material. A disadvantage is that it introduces abundant new coined terms into micromorphology which already is in danger of having too many.

Techniques used in detailed description now include morphometric, chemical (such as the use of sodium hypochlorite), and optical including polarised light, phase contrast, dark field, incident light and fluorescence in U.V. light (Babel, 1971b).

Humus Forms

There has been little detailed work on specific humus forms, except two studies of moder (Bal, 1970; Babel, 1971a, 1972b) and experimental studies of mull formation by earthworms (Jeanson, 1967, 1971, 1972a, 1972b).

Bal analysed the fauna from two moder humus profiles, one under red oak and the other under Douglas fir. The study included a macromorphological description of the humus profiles, orientational faunal analysis to determine the constituent fauna, the culture of the most important faunal inhabitants and quantitative determination of the various components in thin section. Diptera larvae were the main devourers of red

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oak litter and Ptiracaridae of Douglas fir litter. Oak litter was consumed faster than fir litter and the horizons formed under the two types of litter differed in thickness. There was also a marked difference in the durability of the excrement that the two litters contained. Excrement derived from fir was more durable than that of oak and had a well marked ageing process which excrement of oak material lacked.

Babel (1971a, 1972b) studied stages in litter breakdown in four moder profiles under beech, oak and mixed woodland. He described each profile macromorphologically and made a fabric and particle size analysis of each horizon. Mechanical disintegration of plant fragments was mainly by soil animals, with little by microorganisms and virtually none by abiological activity. Lignin and most cellulose decomposed rapidly but some cellulose was preserved by cutin and suberin for several decades. The most important substances forming stable humus were leaf browning substances (protein-tannin complexes). In the early stages of mechanical disintegration there was a laminated fabric whereas in the final stages the fabric consisted of loose excrement or a dense arrangement due to welding together of excrement, other organic remains and mineral grains. Organic substances were blended into the mineral soil by gravity and rainwater and to a lesser extent by soil animals.

These two careful studies have provided a more detailed picture of the inter-relationships between morphology and processes in moder humus. In particular the effects of type of litter and fauna on the morphology of the humus profile have been demonstrated and there is now a better understanding of the processes leading to the division of moder humus into L, F and H layers.

Jeanson (1967, 1971, 1972a, 1972b) examined the role of earthworms in the development of soil structure, mixing of the soil and in the formation of mull. She conducted laboratory experiments in which earthworms were added to an artificial soil consisting of material from a B horizon of a loess-derived soil, calcium carbonate and chopped straw or lucerne. This simulated conditions common in arable soils in Western Europe in which crop residues are ploughed into the soil.

Stages in the modification of the soil material were monitored and the direct effect on the physical properties of the material and indirect effect of earthworm activity on the physico-chemical properties were observed. The soil environment, particularly the pH and content of organic matter and calcium carbonate had a large effect on earthworm activity. Most modification and structure formation occurred in zones with a high reaction (pH 7-8) rich in organic matter and calcium carbonate. Fermentation of lucerne remains in pockets created a moist, reducing environment and calcium and iron migrated to better aerated areas around the channels left by earthworms. Humic substances deposited by earthworms also coated the walls of the channels.

Microflora and Fauna

In recent years, because of a strong feeling among microbiologists that culture experiments give an incomplete and biased picture of the soil microflora, there have been several attempts to observe the microflora directly using various techniques of microscopy including thin sections and scanning electron microscopy. Nicholas et al., (1965) investigated fungi in podzols by means of thin sections and assessed seasonal changes in the population by measuring the length of the fungal mycelium per unit amount of soil. Six morphological groups of mycelium could be recognised but the problem remains of identifying the organisms seen in thin section. Attempts to study bacteria in thin sections have been less successful mainly because unstained bacteria cannot be easily seen. Some success has, however, been obtained with the study of *Bacillus subtilis* and *B. circulans* (Hill and Gray, 1967).

Gray (1967) used the scanning electron microscope to examine fungi and bacteria 'in situ' on the surfaces of the soil particles. He found that mineral and organic particles from a sand-dune soil had associated with them a variety of fungi and bacteria which could be identified at high magnifications. Further studies of soil microflora in a variety of habitats were made by Hagen et al., (1968), Klainer and Betsch (1970) and Todd et al., (1973).

Much information concerning the character of plant litter has been obtained from the studies of moder and mull outlined in the previous section. In addition, there have been other studies that have considered the role of soil fauna in decomposition of plant litter and soil formation as a whole. Thus Zachariae (1965, 1967a, 1967b) studied the role of soil fauna in the decomposition of beech and spruce litter and divided the decomposition of beech litter and eleven stages each characterised by a certain group of fauna. The number of stages in any one soil depended on the habitat.

The role of *Bibio* larvae in the formation of a rendzina from Hungary was investigated by Szabo et al., (1967). The larvae were estimated to consume approximately fifteen per cent of the plant litter and the total amount of excrement produced by these organisms in a year corresponded to 0.04 per cent of the soil matter.

Sleeman and Brewer (1972) examined the materials in termite nests and distinguished three types of materials based on proportion of mineral and organic matter present. Within each of these, sub-classes were distinguished on patterns of voids, occurrence of organic nodules and laminae and different fabric types. These morphological features were related to species, feeding habit, part of nest and location of nest.

From these studies of soil fauna it has been possible to characterise the various types of faunal excrement and Bal (1973) has classified forms of excrement by their shape, size, internal fabric and distribution patterns. From the feeding habits and excrement type of various fauna it is possible in many cases to determine in thin sections the part they play in the decomposition of plant material. In others it is difficult because the excreta of some fauna is of two or more different sizes and shapes. Furthermore, recognition of excrement types is often only possible while the excrement is fresh although some with a well marked ageing characteristic can be identified even when partly decomposed (Zachariae, 1965; Babel, 1968; Bal, 1970).

Humus Chemistry and Micromorphology

Several authors have attempted to relate morphological features of organic matter to humus chemistry; this field has increased in popularity in recent years. Parfenova and Yari-lova (1967) correlated humus microfabrics with organic matter content and composition in three zones of the European U.S.S. R. In the steppe zone chernozems have dark coloured humus, with a humic/fulvic acid ratio of 1.5-2.0. The humus is present partly as strongly flocculated dark brown clusters (1-3 μ diameter), possibly of pure organic matter and a less flocculated lighter coloured material associated with the clay. In the chestnut soils in the same zone light coloured humus associated with the clay predominates and the humic/fulvic acid ratio drops to 1.2-1.4 in the light coloured chestnut soils.

Grey forest soils in the forest-steppe zone contain both dark coloured, flocculated mull humus and various forms of dispersed humus, e.g. organans. Humus in the surface horizons has a humic/fulvic acid ratio of 1.0. Podzolic soils in the forest zone have more fulvic acids than other soils of other zones and there is little or no association of humic acids with calcium.

In a more detailed study of chernozems, Yarilova (1972) related the dark coloured, more or less rounded aggregates of humus to humic acids in which most condensed aromatic rings are linked to calcium whereas the lighter coloured humus associated with clay consists of humic acids of simpler structure, more easily peptized and mobilised.

Drozd (1970, 1973) and Kowalinski et al., (1973 a,b,c) investigated organic compounds in soils to (i) quantitatively and qualitatively determine their nature in various soils under different land use; (ii) determine the relationship between the composition of various humus fractions and certain components from different horizons within the profiles; and (iii) provide a more detailed insight into the character of humic acids isolated from horizons of accumulation in various soils under different land use.

By sequential extractions with $\text{Na}_4\text{P}_2\text{O}_7$, NaOH and a combination of both organic matter was divided into four categories based on the strength of bonding with mineral matter. The authors also examined the micromorphological characteristics of the soils thus making it possible to relate the composition of the various humus fractions with micromorphological features. Clay/organic matter associations are common in soils but so far have proved difficult to characterise micromorphologically.

Jacob (1973) made a detailed study of organic substances in the F layer of an *Auflage* humus and microscopically distinguished three groups of material, humin substances, coal-like substances and slightly changed vegetable bitumens, which in addition to differing optically are also different chemically.

Translocation and Re-distribution of Organic Matter

De Coninck and Righi (1969) considered that translocation of organic matter in podzols is often a twofold process involving a phase of biological transport by burrowing animals and a second phase with migration of dispersed humus. Under wet conditions biological transport is dominant whereas under dry conditions both processes are important. The pelletty fabric is attributed largely to faunal excrements or their remains and the coatings around grains to translocation of dispersed humus from horizons above.

Jongerijs (1970) defined regrouping of soil components as including all changes in distribution and orientation patterns not involving additions or transformations of mineral components; this is accepted as an important phenomenon in soils. Regrouping phenomena include pedoturbations (mixing of components by natural processes and by human activity), pedocompactions (stress-induced phenomena) and concentrations (e.g. of illuvial clay or humus). Several regrouping phenomena involve organic matter, e.g. in slaking of topsoils there is sorting of components, formation of cutans of mixed mineral/organic materials, etc. In Knip soils, the other situation chosen by Jongerijs to illustrate the regrouping concept, transfer of

topsoil material by gravity through cracks into subsurface horizons is an important process, as it is in most soils prone to cracking.

Application of Micromorphology to Problems of Soil Cultivation

In recent years micromorphology has been applied to practical problems associated with soil cultivation and the type of agriculture practised. Barratt (1967, 1968b) studied humus forms and their microfabrics as induced by long-term top dressings in hayfields. Surface matting occurred in the untop-dressed plots and the microfabric was a mullicol-argillicol intergrade. Regular additions of ammonium sulphate, which causes acidification, tended to eliminate earthworms in particular, further degrading the type of humus, with the result that a laminated mor with humuskel microfabric developed. Sodium nitrate and topdressings of lime and basic slag which raised the pH to about six improved most humus forms, particularly if topsoil casting earthworms were present.

The possible deleterious effect of superphosphate additions to compost have also been noted (Altemüller and Tietjen, 1967). With the addition of 1 per cent of superphosphate a large amount of organic material converted to coprogenic aggregates of organic and mineral matter but with 2.5 and 5.0 per cent additions of superphosphate there is much less animal activity and castings are essentially pure organic matter and the mineral part remained dense and structureless.

Barratt (1968a) studied humus forms under different types of land use in Britain and New Zealand. Conversion of forest to pasture decreased the concentration of organic matter near the surface, the granularity of structure and the interpedal porosity but topdressings reversed these trends and granular mulls with spongy mullicol microfabric developed.

Pesticides can have a similar effect to acidifying fertilizers in promoting development of laminated mor with a humuskel microfabric. Thus treatment of orchard soils with copperoxychloride or DNOC was shown by Van de Westeringh (1972) to cause a decline in earthworms and a build-up of plant litter on the surface. Thin sections of the topsoil showed an

absence of earthworm casts and a small proportion of biogenic pores.

The role of microorganisms in stabilising soil structure has been investigated at Rothamsted (Rothamsted Annual Report, Part I, 1972). Soil structure is markedly improved by the addition of 'whey', a by-product of protein extraction, and thin sections show that this phenomenon is due to microbial polysaccharides associated with *Clostridium butyricum* and *C. pasteurianum* forming cutans around most voids.

In Hungary, micromorphology has been used to determine the optimum depth of placement of organic manures in sandy soil (Szabo and Egerszegi, 1972). Four phases in the transformation of the manure are recognised. In the juvenile phase (1-2 years), the organic layer is densely interwoven by roots and there is much microfloral and faunal activity. The mature phase corresponds to an increase in density of roots with transformation of organic material taking place mainly in the rhizosphere. Organic material not in contact with the rhizosphere retains its original cell structure for several decades suggesting inhibited microbial activity. In the senile phase, boundaries of the organic manure become diffuse and microbial and faunal activity is small. The final phase is characterized by homogenisation and dispersion of resistant organic remains and artificial stratification is eliminated completely.

Micromorphological methods of determining the decomposition rate of organic matter have been compared with field assessments and physico-chemical methods by Kowalinski and Kollender-Szych, (1972). They found that the micromorphological method is the best for the qualitative determination of the different stages of decomposition and humification, but that in well-decomposed muck-soils a high magnification together with physico-chemical methods are necessary to obtain reasonable results.

Techniques in Thin Section Preparation and the Study of Organic Materials

Few new preparation techniques have been developed and

polyester and epoxy resins, polyethylene glycols, agar and gelatin remain the chief impregnating media. All have some disadvantages. The use of polyester and epoxy resins necessitates drying the samples before impregnation with the concomitant possibility of altering structure through shrinkage, though this can be alleviated to some extent by freeze drying. Polyethylene glycols (e.g. Carbowax) have the disadvantage that they are crystalline and optically anisotropic, and the impregnated material cannot easily be sectioned mechanically. Samples impregnated with agar and gelatin must be cut with a microtome, limiting the minimum thickness of sections obtainable, particularly if mineral grains occur in the sample. Microbiologists have recently become more interested in the optical examination of undisturbed specimens. Methods suitable for the study of fungi has been reviewed by Nicholas and Parkinson (1967) and for the study of bacteria by Gray et al., (1968).

Staining is important for the study of fungi and bacteria in thin sections. Resin-impregnated sections are generally too thick for unstained bacteria to be seen. Stain has to be introduced before resin impregnation and this often disturbs the structure. Agar and gelatin sections are superior in this regard because stain can be applied after the sections are made. Fluorescent antibody techniques have been applied with some success to the study of bacteria (Unger and Wagner, 1965; Hill and Gray, 1967). In this method, antisera to known bacteria isolated are prepared and the antigen coupled with a fluorescent dye. The soil is then treated with a fluorescent antibody, complexes between the antibody and antigenic bacteria are formed and located by the fluorescence.

There are still several limitations to the use of thin sections for studies of fungi and bacteria. With fungi, organisms are difficult to identify and there is no satisfactory technique to determine the viability of the organisms (Nicholas and Parkinson, 1967). Because most bacteria are attached to surfaces of particles and do not occur as commonly in interparticle spaces, the chance of the section cutting through a particle at points where bacteria are present is remote (Gray et al., 1968).

Because of these problems, microbiologists have turned to transmission and scanning electron microscopes. The scanning electron microscope was used by Gray (1967) to examine microorganisms on the surfaces of soil particles and roots, and several studies used the transmission electron microscope (Nikitin, 1964, 1973; Harris, 1972; Greaves and Darbyshire, 1972; Foster and Rovira, 1973; Waid, 1973).

Babel (1972a) used fluorescence microscopy for the morphographic study of organic substances. Tissue and cell wall residues, hyaline hyphae and fungal spores are examples of organic material that become visible and identified through primary fluorescence. As humification proceeds organic particles lose their fluorescence.

Jacob (1972) attempted to characterise organic material in soils by measuring fluorescence intensity and reflectivity; morphography and this technique has also been used to examine organic substances in the fermentation layer of Auflagehumus (Jacob, 1973).

SUMMARY AND OUTLOOK

In the past decade there have been several changes of emphasis in the study of the micromorphology of organic matter. The early part of the period saw a continuation of the work on humus forms, description and classification systems and techniques, but in recent years despite a large increase in the number of micromorphologists there has not been a corresponding increase in work on these aspects. Several important humus forms, particularly peat and raw humus, have received little attention and it is hoped that in the next few years these will be given the same standard of treatment that moder and mull have received.

Somewhat compensating for the lack of work on some humus forms there has been a marked increase in the application of micromorphology to agricultural problems, and papers have been published on the effects of fertilizers on humus forms and soil fauna and on the optimum placement of organic manures. This aspect is likely to receive even more attention partly

because there is increasing appreciation of the role that micromorphology can play in helping to solve problems associated with agriculture and forestry and partly because many micromorphologists are in research institutes where the need for solution of these problems is likely to be felt.

Some fields largely neglected earlier have in recent years been given more emphasis. There have been several attempts to study humus chemistry in relation to morphological features in thin section and through this work there is a better understanding of the stability of plant substances, the relative breakdown of plant material and the formation of new compounds. There still remains, however, the difficulty of identifying organic compounds in thin sections.

The scanning electron microscope has introduced a new dimension into soil micromorphology. It has been used by microbiologists to study fungi and bacteria and by soil zoologists to study soil fauna and the redistribution of materials by earthworms. So far it has been little used in studying the breakdown of plant litter although for this it would be an extremely useful tool.

With increasingly sophisticated instruments, a more quantitative approach has been possible in the study of soil micro-heterogeneity. The microprobe analyser used with the scanning electron microscope provides valuable quantitative data on element distribution and measurements (e.g. size, shape, area, perimeter) of features. Such studies which previously were very laborious and hence rarely done, can now be made more rapidly using the image analysing computer.

In addition to the study of micromorphology of organic matter *per se*, it is hoped that in the next few years micromorphology will be used regularly in investigations combining several methods of characterisation. This will enable information obtained from macromorphological, micromorphological, chemical, biological and physical methods to be linked, an approach that will represent an important step forward.

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